Journal of Horticultural Science & Biotechnology (2003) **78** (6) 798–812

Soil pasteurization and inoculation with *Glomus intraradices* alters flower production and bulb composition of *Zephyranthes* spp.

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SUMMARY
We assessed whether adding inoculum of the vesicular-arbuscular mycorrhizal fungus (VAMF) *Glomus intraradices* into growing medium of three *Zephyranthes* spp (White Rain Lily [WRL], *Z. candida*; Pink Fairy Lily [PFL], *Z. robusta*; Yellow Zephyr Lily [YZL], *Z. sulphurea*) alters aspects of flower and bulb production. Shoots of inoculated plants emerged 7–13 d earlier than those of non-inoculated plants. Inoculation slightly delayed the emergence of flower buds on WRL and PFL, but did not delay the time of flower opening of WRL. Inoculated YZL flowered 4–11 d earlier than non-inoculated plants. The number of flowers produced by YZL was consistently increased by inoculation, while the inoculation with VAMF increased flower production by WRL and PFL only when plants were growing in pasteurized soil. Leaf biomass of inoculated WRL was larger than non-inoculated plants, while leaf biomass was generally smaller in inoculated PFL and YZL. Partitioning of biomass to bulbs and offsets varied with species, soil pasteurization, and inoculation. Inoculation increased the combined weight of bulbs and offsets at the end of the second growing cycle by 50–150%. Inoculated YZL and WRL consistently produced more offsets in the second growing season after inoculation. For all species, inoculation increased phosphorus and carbohydrates and decreased nitrogen and amino acids in bulbs. Adding VAMF into the growing medium of *Zephyranthes* altered aspects of plant development and biomass partitioning important to flower and bulb production during the first growing cycle after inoculation, and most effects of VAMF inoculation are more pronounced in the second growing cycle after inoculation. Of the three species examined, *Z. sulphurea* showed the most consistent responses to inoculation.

Plants with roots colonized by vesicular-arbuscular mycorrhizal fungi (VAMF) are more effective at nutrient and water acquisition, less susceptible to disease, and can be more productive under certain environmental growing conditions than plants without mycorrhizae (Smith and Read, 1997). There is little available information describing the benefits of inoculation with mycorrhizal fungi on different aspects of productivity and flowering of lilaceous bulb crops except onion (Tawaraya *et al.*, 1999) and Easter lily (*Lilium longiflorum*) (Ames and Linderman, 1978; Mora, 1990). The influence of VAMF inoculation on productivity of different crops has been well documented, however few reports detail differences carbon and nutrient allocation patterns and flowering between mycorrhizal and non-mycorrhizal plants (Bryla and Koide, 1998). The VAMF fungus *Glomus intraradices* has been reported to decrease the number of days until flower opening, increase the number of flowers per inflorescence and flower longevity, and alter biomass partitioning between corms and cormels of *Brodiaea laxa* (*Triteleia laxa*) 'Queen Fabiola' corms (Scagel, 2003). Carbon and nutrient allocation patterns can influence bulb quality of liliaceous ornamentals. Bulb quality can, in turn, affect new bulb formation and flower production and is influenced by several factors during cold storage (temperature, moisture) and during the growing season (light, nutrients, temperature) (Han *et al.*, 1991).

The genus *Zephyranthes* in the *Amaryllidaceae* contains several species of flowering bulbs used in landscape and potted flower production (Smith *et al.*, 1999). *Zephyranthes* spp. grow from truncated bulbs, with their active period of growth and flowering in the summer and a rest period in the winter (De Hertogh and Le Nard, 1993). Flower formation in this genus occurs alternately with leaf formation during the whole assimilation period. Propagation of *Zephyranthes* spp. generally occurs by offset bulblets. The objective of this study was to determine whether addition of VAMF inoculum into the growing medium of different *Zephyranthes* spp. alters aspects of flower production, bulb production, and bulb quality.

MATERIALS AND METHODS

Plant material and treatments

Bulbs of White Rain Lily (*Zephyranthes candida* (Lindl.) Herb.), Pink Fairy Lily (*Z. robusta* (Herb. Ex Sweet) baker syn *Habaranthus robustus* Sweet), and Yellow Zephyr Lily (*Z. sulphurea* syn. *Z. citrina* Baker) were planted into cylindrical 3.78 l pots (Lerio 19.4 cm $\times$ 18.1 cm) containing either a steam pasteurized (60°C for 30 min) or a non-pasteurized 1:1 mixture of a Willamette Valley alluvial silt loam and river sand (11 mg kg^{-1} available (Bray) phosphorus (P), pH of 6.3). For the VAMF treatment, inoculum of the VAM fungus (*Glomus intraradices* Schenck & Smith) at a rate of 1:166 (v/v) was placed beneath the base of each bulb at planting. For controls, sterilized inoculum was added at the base of each bulb at the same rate.

Mycorrhizal inoculum

Glomus intraradices Schenck & Smith was originally obtained from Native Plants Incorporated, (Salt Lake City, Utah) and maintained in pot cultures at the USDA-ARS, Horticultural Crops Research Laboratory in Corvallis, Oregon. The fungus was propagated in pot cultures on roots of bunching onion (*Allium cepa* L. 'White Lisbon') grown in 1:1 loam:sand for five months. Inoculum consisted of a mixture of the soil medium, extraradical hyphae and spores, and colonized root segments (<2 mm in length). Population estimates of the inoculum used in this study by the MPN method (Woomer, 1994) averaged 10 propagules g^{-1} of soil.

Cultural conditions

Plants were maintained in a glasshouse with supplemental light (16 h/8 h, light/dark) provided by high pressure multi-vapour lamps with an average of 700 μmol m^{-2} s^{-1} at canopy level, and average day/night temperatures of 18/15°C. Plants were fertilized once a week with 50 ml of a liquid fertilizer (LF) (approximately 10% K, 10% P, 40% N, 20% Ca, 7% Mg, 8% S, 4% Na, and less than 0.05% of Mn, Cu, Zn, B, and Mo) and watered as needed. Periodic pest and pathogen control measures were performed as needed in the greenhouse and included diflubenzuron for fungus gnats (*Bradysia* spp.), *Neoseiulus fallacis* predators for spider mites (*Tetranychus* spp.), and *Neoseiulus cucumeris* predators for thrips (*Frankiniella* spp.). At the end of the growing cycle, when stems had died-back, bulbs were removed from the soil, dried at 20°C for two weeks, then stored at 18°C for ten weeks until planting. Bulbs were planted into cylindrical 3.78 l pots (Lerio 19.4 cm [$7\frac{5}{8}$ in] × 18.1 cm [$7\frac{1}{8}$ in]) containing a 1:1 mixture of a Willamette Valley alluvial silt loam and river sand. Plants were grown under the same growing conditions for both the first and second growing cycles. To assess carry-over effects from the first growing cycle, no VAMF inoculum was added for the second growing cycle and all soil was pasteurized as previously described.

First growing cycle measurements

For each bulb the number of days after planting until shoot emergence and flower emergence was recorded. The number of flower buds and the total number of flowers on each plant was recorded. At the end of the growing cycle, shoots were removed from plants by cutting at soil level, oven dried at 60°C, and weighed. Bulbs and offset bulblets (offsets) were removed from the soil, counted, air dried, and weighed. VAMF colonization of fresh roots was assessed on 1 cm sections after clearing and staining by modified procedures of Phillips and Hayman (1970), replacing lacto-phenol with lacto-glycerin. Percentage of root length with signs of VAMF colonization was estimated by the method of Biermann and Linderman (1980). Six daughter bulbs per treatment were placed in storage under the second growing cycle. A subsample (six daughter bulbs per treatment) were analysed for phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu), boron (B), zinc (Zn), carbon (C), nitrogen (N), and sulphur (S) content using standard methods (Gaulak *et al.*, 1997). N and S were determined after automated combustion and concentrations of the remainder of the elements determined after dry ash oxidation by ICP-AES. Total soluble protein was determined colorimetrically using a BIO-RAD (Coomassie Brilliant blue) (Bradford, 1976) assay after extraction of ground bulb tissue (<50 mesh) in buffer (20 mM TRIS, 10 mM NaCl, 10 mM KCl, 2 mM $MgCl_2$ $6H_2O$) with Nonidet P-40. Total amino acid content of bulbs was determined colorimetrically with ninhydrin (Yemm and Cocking, 1955) after extraction with acetic acid prior to analysis. Total reducing and non-reducing sugar contents of bulbs were determined colorimetrically using a modification of the Somolgyi-Nelson Alkaline Copper method (Dische, 1962). Supernatant from ground bulb tissue (<50 mesh) extracted with warm 80% ethanol was used to determine total reducing sugar content. The residual pellet from extraction was hydrolyzed in 0.2N KOH prior to analyses for non-reducing sugars.

Second growing cycle measurements

For each bulb the number of days after planting until shoot emergence and flower emergence was recorded. The total number of flowers per inflorescence and flower longevity on each plant was also recorded. At the end of the growing cycle, when stems had died-back, bulbs and offsets were removed from the soil, counted, air dried, and weighed.

Experimental design and statistical analyses

The experiment was set up in a randomized design with each treatment unit (pot) replicated 12 times during the first year and six times during the second year. Morphological data were subjected to Analysis of Variance (ANOVA) with plant species (S), soil pasteurization (P), and VAMF inoculation (V) and growing cycle (Y) as main effects. Bulb composition data were subjected to three-day Analysis of Variance (ANOVA) with plant species (S), soil pasteurization (P), and VAMF inoculation (V) as main effects. Single degree of freedom contrasts were used to address specific questions comparing species responses to VAMF inoculation when grown in pasteurized or non-pasteurized soils. Data with unequal variances between treatment groups were log-transformed to equalize variances. Back-transformed data are presented in tables. All data analyses were performed using the Statistica® statistical package (Statsoft Inc., Tulsa, OK, USA, 1996).

RESULTS

Plant development

The number of days until shoot emergence after planting varied greatly between the three *Zephyranthes* spp. Differences in shoot emergence was significantly influenced by soil pasteurization (S × P interaction $P<0.0077$) and inoculation with VAMF (S × V interaction $P<0.0384$). Shoots of White Rain Lily (WRL) emerged approximately 30 d after planting, while shoots of Pink Fawn Lady (PFL) and Yellow Zephyr Lily (YZL) emerged approximately 46 and 62 d after planting, respectively (Figure 1). Soil pasteurization had no effect on the number of days until shoot

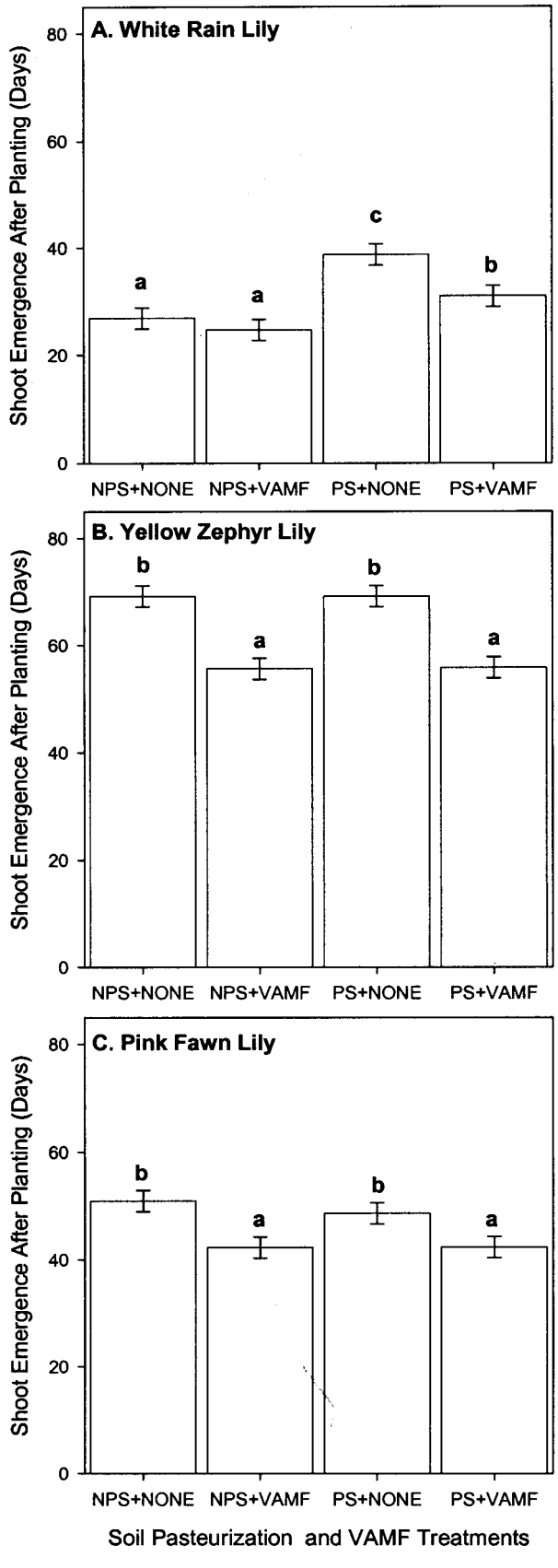

FIG. 1
Influence of soil pasteurization and inoculation with the VAMF *Glomus intraradices* on number of days until stem emergence after planting of three *Zephyranthes* spp. (A) White Rain Lily, (B) Yellow Zephyr Lily, and (C) Pink Fawn Lily. Error bars are SEs of the least squares means over two growing cycles. Columns with the same letters above them within a species are not significantly different ($P<0.05$).

emergence after planting of PFL or YZL but shoots of WRL growing in pasteurized soil emerged 7 d later than shoots of WRL growing in non-pasteurized soil (Figure 1). VAMF inoculation generally decreased the number of days until shoot emergence of all three *Zephyranthes* spp. tested (Figure 1). Inoculation of White Rain Lily (WRL) and Pink Fawn Lily (PFL) with VAMF decreased the number of days until shoot emergence after planting by approximately 7 d, while inoculation of Yellow Zephyr Lily (YZL) increased the number of days by approximately 13 d.

The influence of VAMF inoculation on the number of days until flower bud emergence after planting ($S \times P \times V$ interaction $P<0.0001$) and shoot emergence ($S \times P \times V$ interaction $P<0.0002$) varied significantly with both plant species and soil pasteurization treatment (Figure 2). Flower buds of WRL and PFL generally emerged at the same time after planting regardless of soil pasteurization or VAMF treatments (Figure 2A and C), however the number of days between shoot emergence and flower bud emergence was generally greater in VAMF inoculated WRL and PFL (Figure 2D and F). When YZL bulbs were inoculated with VAMF, flower buds emerged 13–20 d earlier after planting and 1–8 d earlier after shoot emergence depending on soil pasteurization treatment (Figure 2B and E).

The influence of VAMF inoculation on the number of days until flower opening after shoot emergence ($S \times P \times V$ interaction $P<0.0001$) and flower opening after bud emergence ($S \times P \times V$ interaction $P<0.0136$) varied significantly with both plant species and soil pasteurization treatment. Delayed flower bud emergence in VAMF inoculated WRL did not significantly affect the time of flower opening (Figure 3A and D). In contrast, VAMF inoculation delayed flower opening after shoot emergence of PFL by approximately 12 d (Figure 3F). Inoculation of YZL significantly decreased the number of days until flower opening after stem emergence by 1–11 d (Figure 3B and E).

Differences in flower longevity between the three species was significantly influenced by soil pasteurization ($S \times P$ interaction $P<0.0433$) and inoculation with VAMF ($S \times V$ interaction $P<0.0280$). Inoculation with VAMF significantly increased the longevity of YZL flowers by approximately 5 d (Figure 4B) but did not have a significant effect on the longevity of flowers on either WRL or PFL (Figure 4A and C). In general the timing of plant and flower development of YZL was more responsive to VAMF inoculation than either WRL or PFL.

Plant growth and flower production

The influence of VAMF inoculation on the number of leaves ($S \times P \times V$ interaction$P<0.0013$), the dry weight of leaves and shoots ($S \times P \times V$ interaction $P<0.0183$), and the number of flowers ($S \times P \times V$ interaction $P<0.0094$) varied with plant species and soil pasteurization treatment. Inoculation increased dry weight of shoots and leaves of WRL, particularly when grown in non-pasteurized soil (Figure 4D), however the number of leaves and flower production of WRL growing in non-pasteurized soil was generally not affected by inoculation (Figure 5A and D). In contrast, PFL growing in

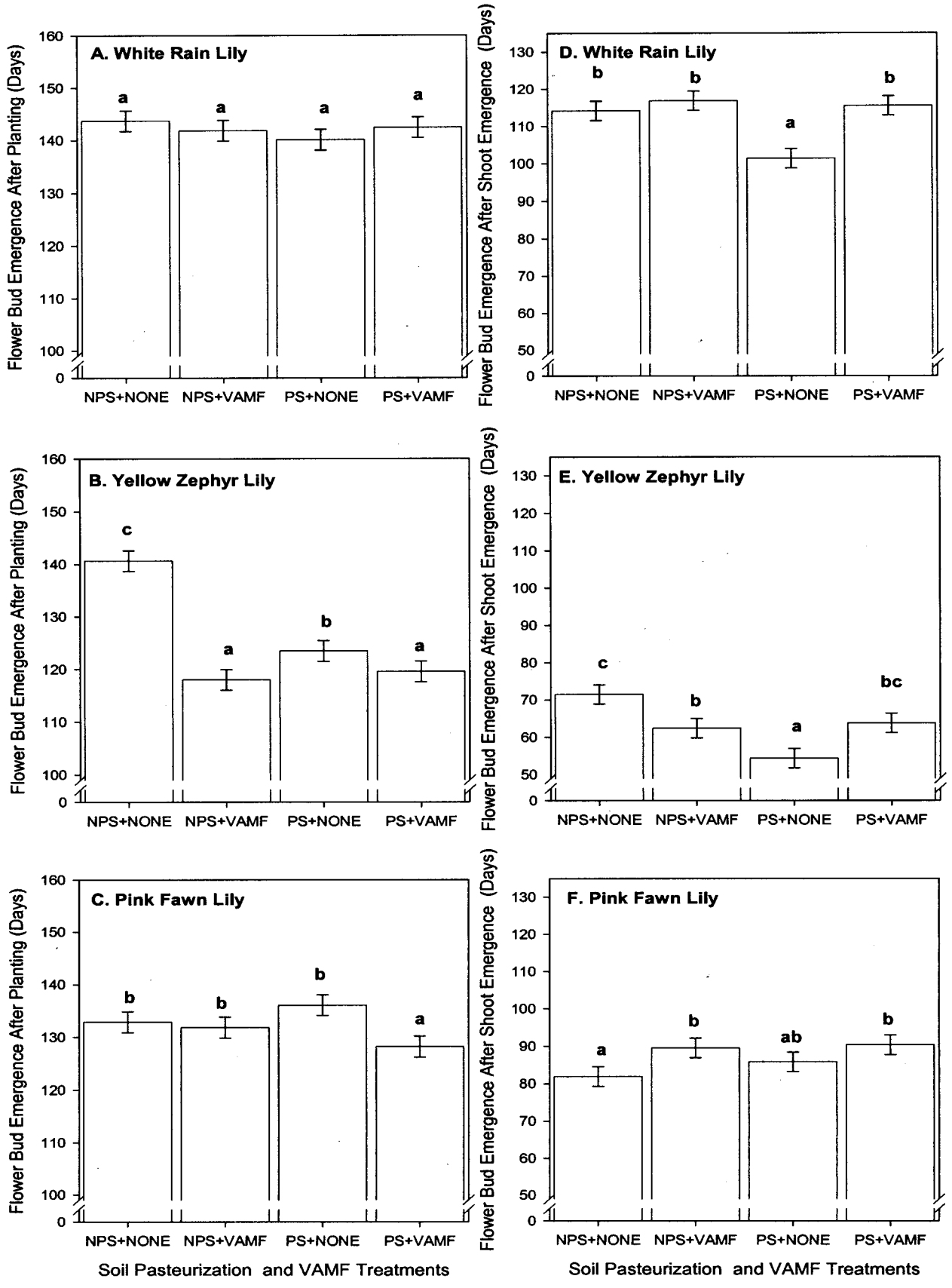

FIG. 2
Influence of soil pasteurization and inoculation with the VAMF *Glomus intraradices* on the number of days until flower emergence after planting (A–C). and stem emergence (D–F) of three *Zephyranthes* spp. (A, D) White Rain Lily, (B, E) Yellow Zephyr Lily, and (C, F) Pink Fawn Lily. Error bars are SEs of the least square means over two growing cycles. Columns with the same letters above them within a species are not significantly different ($P<0.05$).

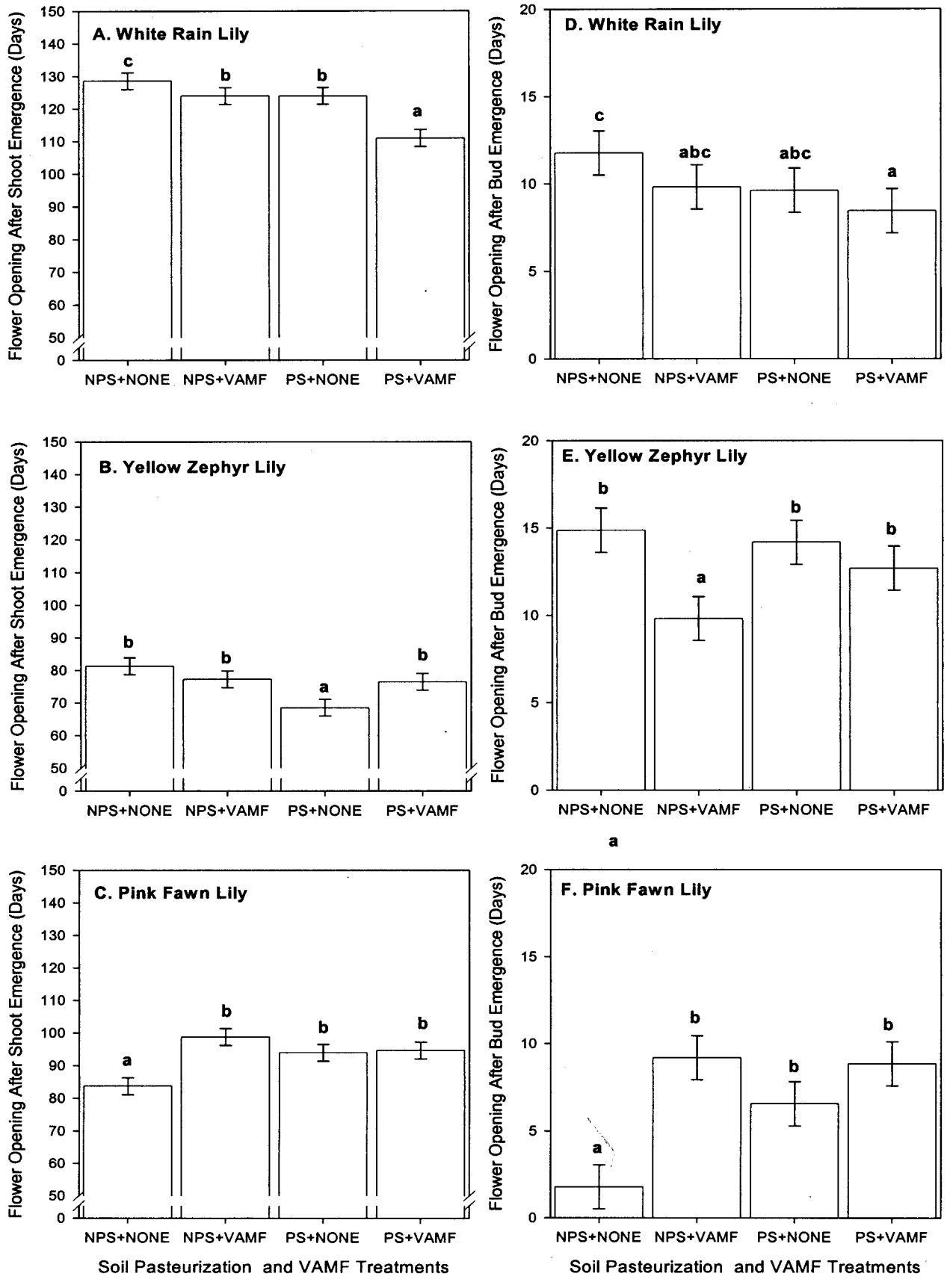

FIG. 3
Influence of soil pasteurization and inoculation with the VAMF *Glomus intraradices* on the number of days until flower opening after stem emergence (A–C) and flower bud emergence (D–F) of three *Zephyranthes* spp. (A, D) White Rain Lily, (B, E) Yellow Zephyr Lily and (C, F) Pink Fawn Lily. Error bars are SEs of the least squares means over two growing cycles. Columns with the same letters above them within a species are not significantly different ($P<0.05$).

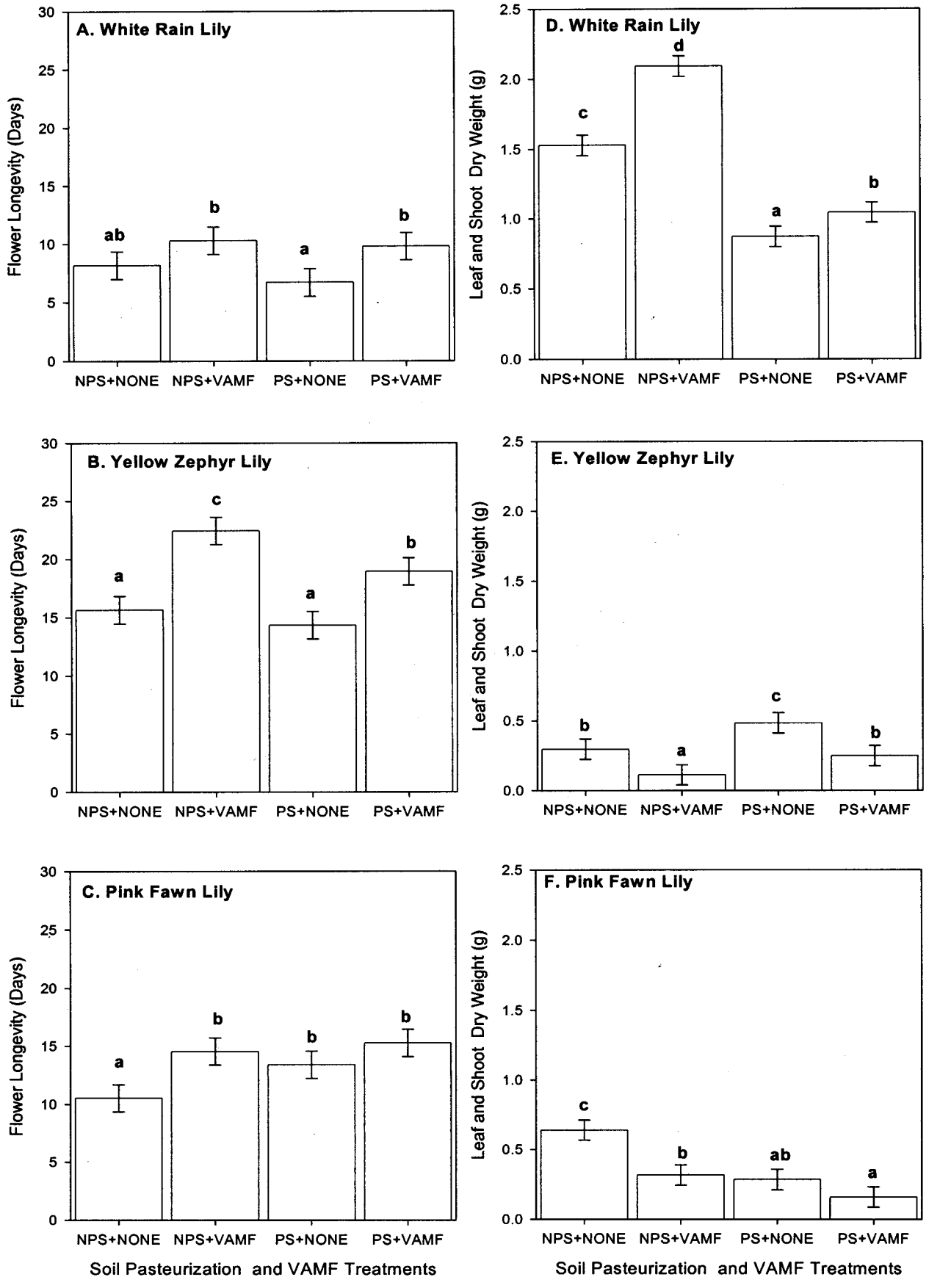

FIG. 4
Influence of soil pasteurization and inoculation with the VAMF *Glomus intraradices* on flower longevity (A–C) and above-ground biomass (D–F) of three *Zephyranthes* spp. (A, D) White Rain Lily, (B, E) Yellow Zephyr Lily, and (C, F) Pink Fawn Lily. Error bars are SEs of the least squares means over two growing cycles. Columns with the same letters above them within a species are not significantly different ($P<0.05$).

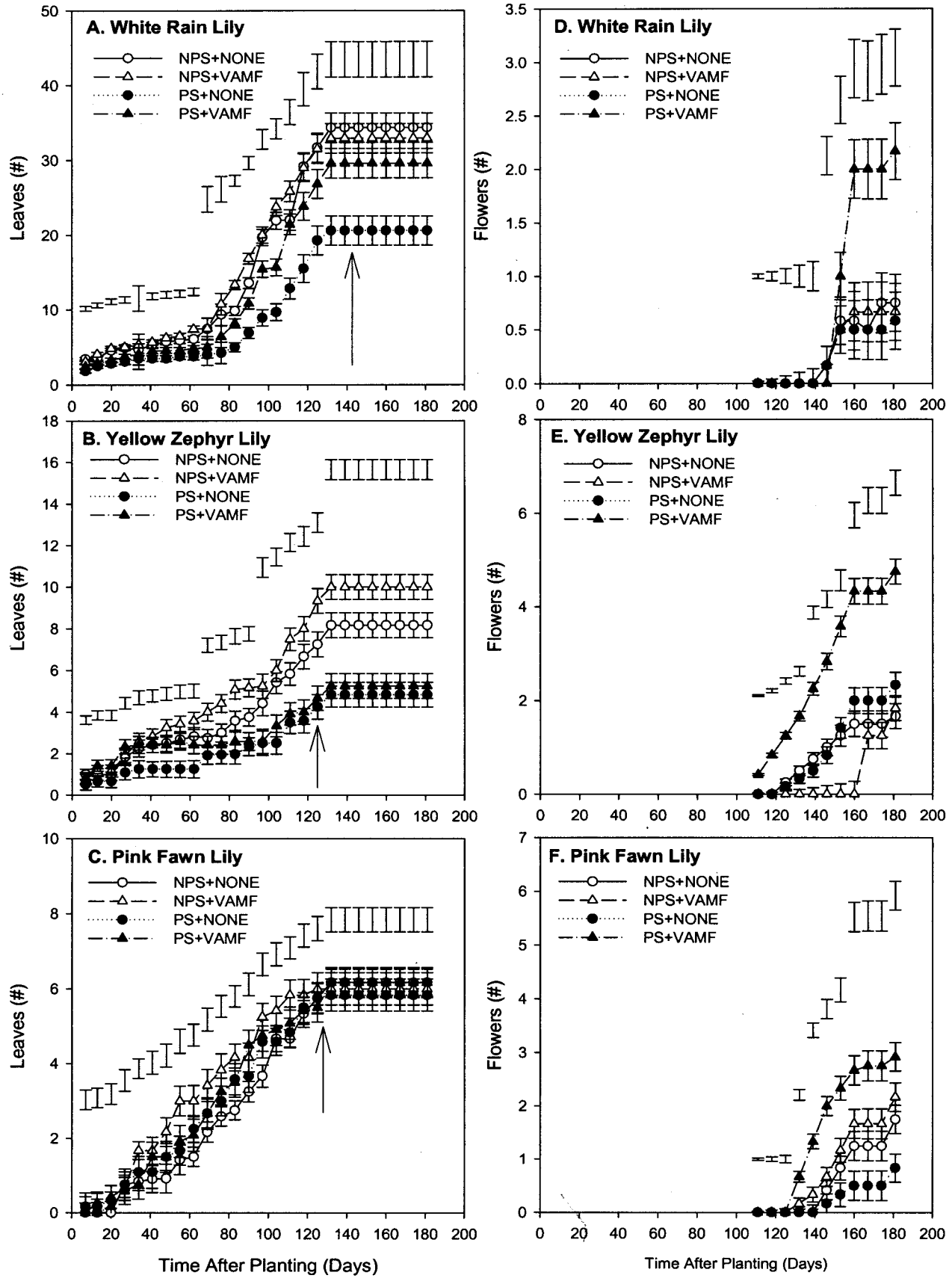

FIG. 5

Influence of soil pasteurization and inoculation with the VAMF *Glomus intraradices* on the total number (cumulative) of fully expanded flowers) (A–C) and leaves of three *Zephyranthes* spp. during the first growing cycle after inoculation and soil treatments (A & D) White Rain Lily, (B & E) Yellow Zephyr Lily, and (C & F) Pink Fawn Lily. Error bars on data points are SEs of the least squares means between measurement dates over two growing cycles. Error bars above data point represents $LSD_{0.05}$ for each measurement date. Arrow indicates average number of days until flower bud emergence for each cultivar.

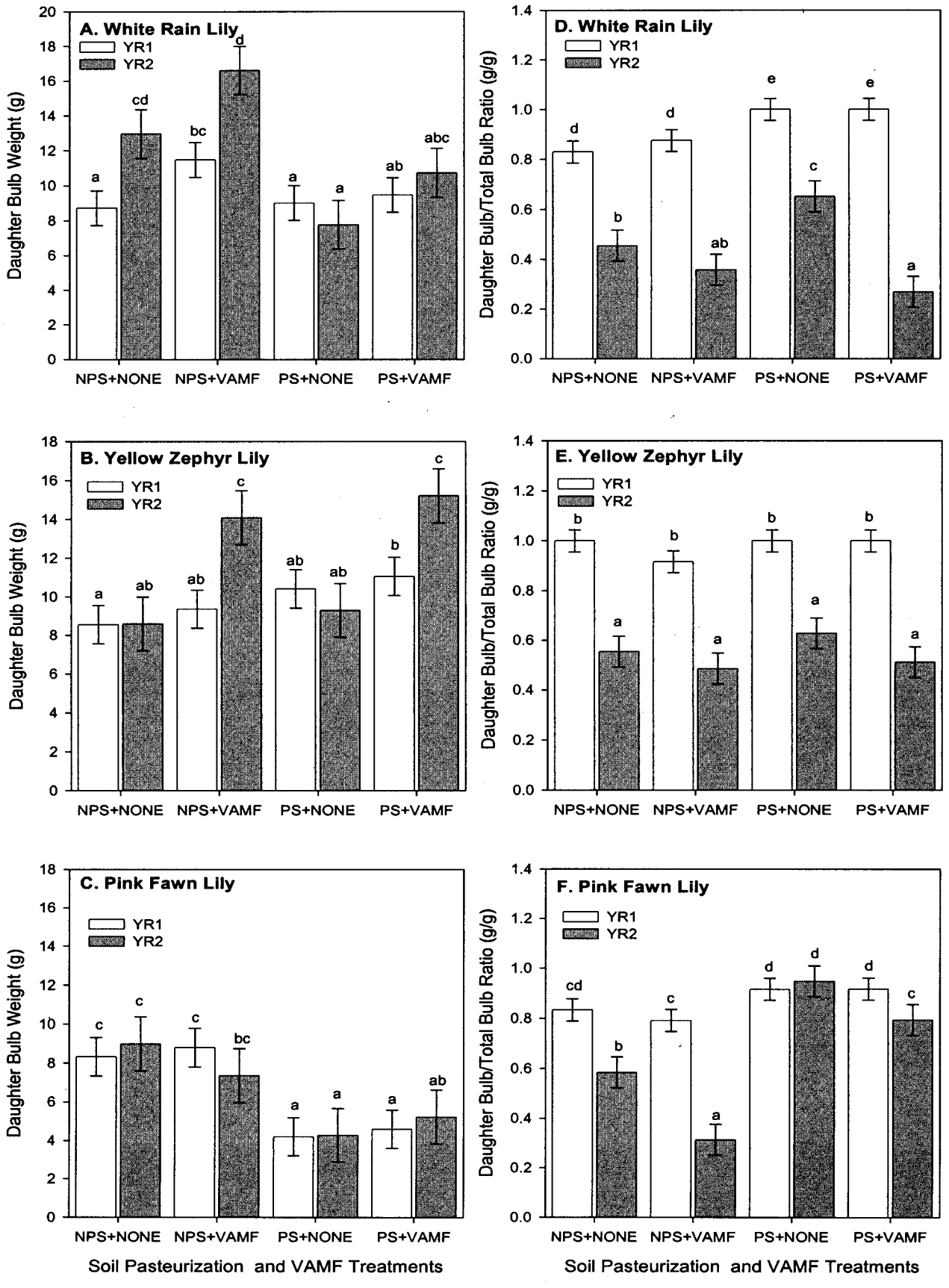

FIG. 6
Influence of soil pasteurization and inoculation with the VAMF *Glomus intraradices* on daughter bulb weight (A–C) and partitioning (D–F) of three *Zephyranthes* spp. (A, D) White Rain Lily, (B, E) Yellow Zephyr Lily, and (C, F) Pink Fawn Lily. Error bars are SEs of the least squares means within measurement dates (YR1, n = 12, YR2, n = 6). Columns with the same letters above them within a species are not significantly different ($P<0.05$).

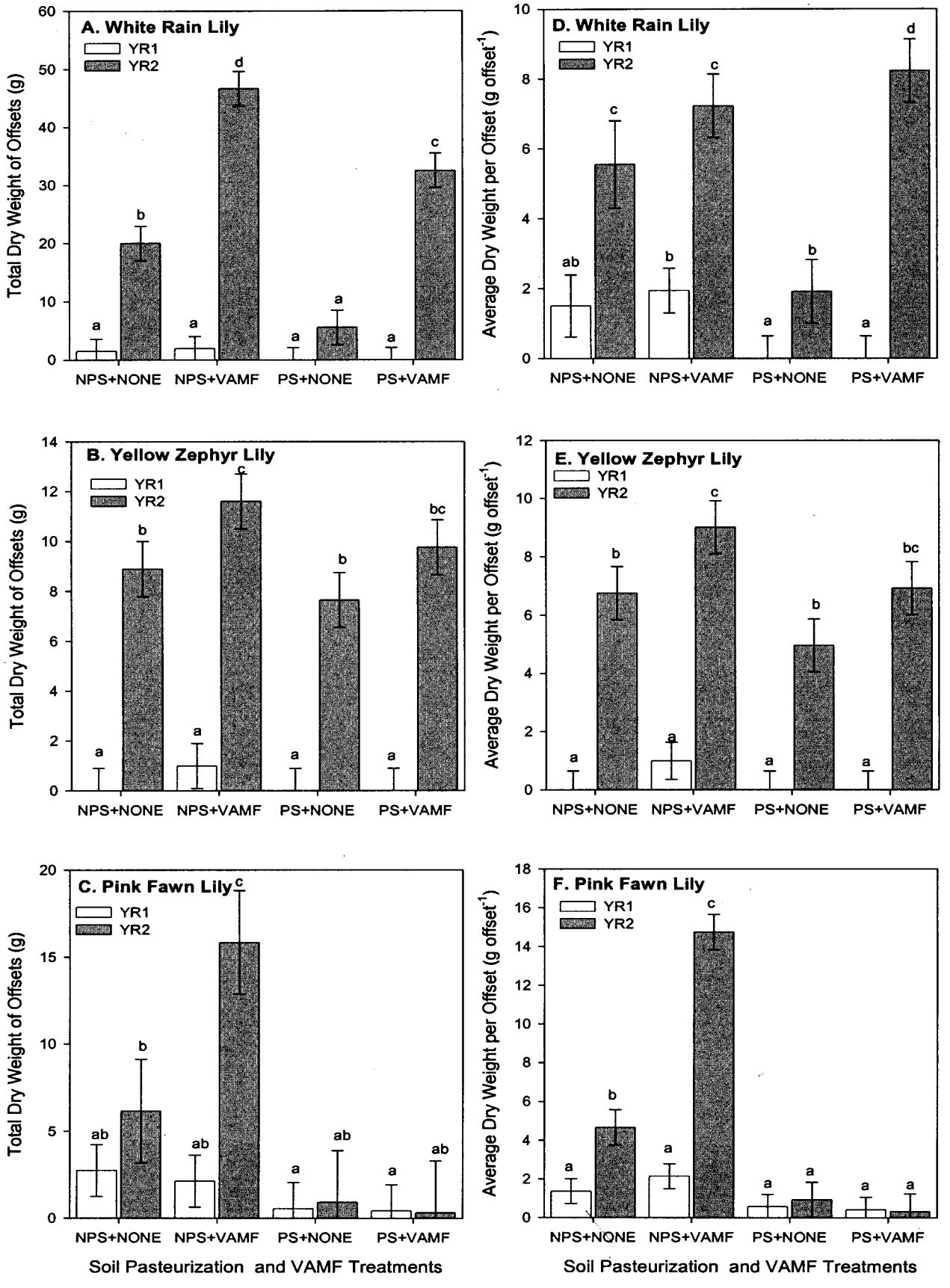

FIG. 7

Influence of soil pasteurization and inoculation with the VAMF *Glomus intraradices* on total offset weight (A–C) and average weight per offset (D–F) of three *Zephyranthes* spp. (A, D) White Rain Lily, (B, E) Yellow Zephyr Lily, and (C, F) Pink Fawn Lily. Error bars are SEs of the least squares means within measurement dates (YR1, n = 12; YR2, n = 6). Columns with the same letters above them within a species are not significantly different ($P<0.05$).

non-pasteurized soil had significantly smaller leaves (lower leaf biomass and more leaves) (Figure 4F, Figure 5C) and delayed flower production (Figure 5F) when compared to non-inoculated plants. VAMF inoculation of YZL also resulted in plants with smaller leaves than those on non-inoculated plants (Figure 4E, Figure 5B, however flower production was not delayed (Figure 5E). In general, the response of leaf production and biomass to inoculation with VAMF was similar in YZL and PFL. We also found that leaf production in both PFL and YZL continued after the start of flower production.

Inoculation of plants with VAMF significantly increased the number of flowers produced per plant, and the magnitude of response varied significantly with plant species and soil pasteurization treatment. Inoculated plants produced significantly more flowers but only when grown in pasteurized soil (Figure 5D, E, and F). Although flowers expanded earlier on inoculated PFL than on non-inoculated plants, the total number of flowers produced was only increased by VAMF when plants were grown in pasteurized soil (Figure 5A, B). Inoculated YZL produced more flowers than non-inoculated plants, regardless of soil pasteurization treatment (Figure 5A, C). For all species, plants stopped producing new flowers at approximately the same time for all soil pasteurization and VAMF treatments.

Bulb and offset production and biomass partitioning

Daughter bulb weight and partitioning of biomass between daughter bulbs and offsets varied between species and was significantly influenced by VAMF inoculation, soil pasteurization, and growing cycle from inoculation. In the first year of our study, VAMF inoculation generally had no influence on daughter bulb biomass (Figure 6A-C). In the second year, the weight of daughter bulbs produced by WRL and YZL was significantly increased by inoculation (Figure 6A and B), but daughter bulb weight of PFL was not significantly influenced by VAMF inoculation (Figure 6C). VAMF inoculation increased the combined weight of bulbs and offsets at the end of the second growing cycle by between 50–150% depending on species and on soil pasteurization treatment (data not shown). Biomass partitioning between daughter bulbs and offsets (ratio of daughter bulb weight to total bulb and offset weight) was not influenced by VAMF inoculation during the first growing cycle after inoculation (Figure 6D-F). In the second growing cycle of our study, inoculated plants partitioned more dry weight to offsets than to daughter bulbs (Figure 6).

Production and size of offsets was highly variable and significantly influenced by VAMF inoculation, soil pasteurization, species, and growing cycle from inocula-

Table I

Influence of soil pasteurization and inoculation with the VAMF Glomus intraradices *on phosphorus (P), potassium (K), Calcium (Ca), Zinc (Zn), Sulfur (S), and nitrogen (N) concentration and content of bulbs from three* Zephyranthes *spp. at the end of the first growing cycle after treatment*

		Concentration (g kg^{-1}, except Zn mg kg^{-1})					
Species[Z]	Treatment	P	K	Ca	Zn	S	N
WRL	NPS+NONE	2.18 a[x]	10.07 b	3.90 c	32.50 c	3.45 c	43.0 d
	NPS+VAMF	2.80 b	10.87 b	3.53 b	33.00 c	2.95 b	35.1 b
	PS+NONE	2.06 a	8.77 a	3.00 a	27.30 a	3.05 b	37.1 c
	PS+VAMF	2.78 b	9.00 a	2.80 a	29.70 b	2.66 a	30.7 a
YZL	NPS+NONE	2.67 a	14.88 c	2.43 b	25.50 b	2.53 a	37.6 d
	NPS+VAMF	3.40 b	16.65 d	2.21 a	27.50 c	2.78 b	31.1 b
	PS+NONE	2.90 a	12.25 a	2.70 c	23.30 a	2.72 b	34.7 c
	PS+VAMF	3.85 c	13.87 b	2.71 c	21.70 a	2.73 b	28.0 a
PFL	NPS+NONE	3.95 a	11.40 b	3.20 b	29.00 c	2.63 b	37.3 b
	NPS+VAMF	4.60 bc	10.93 b	2.43 a	26.70 b	2.37 a	29.5 a
	PS+NONE	4.40 b	9.37 a	4.40 d	28.20 c	3.53 d	49.6 c
	PS+VAMF	4.64 c	8.94 a	3.61 c	23.00 a	2.90 c	38.2 c
(SE)[W]		(0.12)	(0.38)	(0.13)	(1.0)	(0.08)	(0.98)
ANOVA Effects (P<0.05)[V]		Z, S, V	Z, S. V, Z*V	Z, S, V, Z*S, Z*V	Z, S, Z*V	Z, S, V, Z*S, Z*V	Z, V, Z*S
		Content (mg bulb^{-1})					
Species	Treatment	P	K	Ca	Zn	S	N
WRL	NPS+NONE	20.2 a	95.8 b	36.4 b	0.306 b	33.2 b	401.7 c
	NPS+VAMF	33.1 c	129.7 c	42.2 c	0.393 c	25.0 a	417.9 c
	PS+NONE	20.1 a	84.6 a	29.0 a	0.264 a	29.2 b	356.7 b
	PS+VAMF	24.4 b	78.0 a	24.3 a	0.257 a	23.1 a	267.4 a
YZL	NPS+NONE	23.2 a	130.2 a	21.0 a	0.224 a	22.1 a	324.5 b
	NPS+VAMF	33.5 b	162.4 c	21.6 a	0.281 d	27.9 b	306.9 a
	PS+NONE	34.8 b	148.5 b	32.3 b	0.279 c	32.4 c	418.7 c
	PS+VAMF	41.8 c	150.9 b	29.3 b	0.232 b	30.2 c	303.0 a
PFL	NPS+NONE	37.9 b	110.0 c	30.9 b	0.279 b	25.4 c	358.4 c
	NPS+VAMF	58.2 c	136.8 d	30.7 b	0.336 c	29.6 d	367.8 c
	PS+NONE	22.6 a	50.9 b	22.7 a	0.145 a	18.0 b	255.5 b
	PS + VAMF	20.3 a	39.2 a	15.9 a	0.117 a	12.7 a	167.9 a
(SE)		(4.6)	(11.3)	(3.3)	(0.028)	(2.67)	(35.4)
ANOVA Effects (P<0.05)		Z, S, V, Z*S	Z, S, Z*S	Z, S, Z*S	Z, S, Z*S	Z, S, Z*S	Z, S, Z*S

[Z]WRL = White Rain Lily (*Z. candida*), YZL = Yellow Zephyr Lily (*Z. sulphurea*), PFL = Pink Fawn Lily (*Z. robusta*).
[Y]NPS = Non-pasteurized soil; PS = Pasteurized soil; NONE = Sterilized VAMF inoculum, VAMF = VAMF inoculum.
[X]Means followed by the same letter within a species are not significantly different (P<0.05) using single degree of freedom contrasts.
[W]SE across all species (n = 6).
[V]Significant (P<0.05) main effects and interactions from ANOVA where Z = Plant species; S = Soil pasteurization treatment, V = VAMF inoculation treatment, Y = year from inoculation.

tion (Figure 7). Total weight of offsets for all species was generally higher in the second growing cycle after inoculation. Inoculation had no effect on total weight of offsets in the first growing cycle after inoculation but generally increased total weight of offsets during the second growing cycle (except for PFL in pasteurized soil) (Figure 7A-C). Inoculation with VAMF had no influence on the number of offsets produced during the first growing cycle when plants of all species produced, on average, only one offset. Inoculated plants of WRL and YZL produced 2–4 more offsets than non-inoculated plants in the second growing cycle after inoculation while PFL produced an average of one offset in the second growing cycle after inoculation regardless of VAMF treatment. The average weight per offset increased significantly between the end of the first and second growing cycles for all species and in general inoculated plants produced offsets that were heavier than non-inoculated plants (Figure 7D-F).

Mineral composition

Differences in concentration and contents of elements in bulbs in response to VAMF varied with species (Table I). Soil pasteurization had a large influence on total content of all elements in bulbs from all species. We found that the concentrations and contents of P, K and Zn in *Zephyranthes* spp. bulbs at the end of the first growing cycle were generally higher in VAMF than non-VAM inoculated plants (Table I). Nitrogen content in bulbs of VAMF-inoculated plants was generally lower or equal to that found in non-inoculated plants, while N concentrations were lowest in VAMF inoculated plants (Table I).

Protein, amino acid, and carbohydrate composition

We found that inoculation with VAMF decreased protein concentrations in bulbs (Table II), but changes in protein concentration were associated with proportional increases in protein content and bulb weight, therefore inoculation had no influence on protein production and accumulation in bulbs. Concentrations and contents of amino acids and sugars were significantly lower in inoculated bulbs than non-inoculated bulbs of *Zephyranthes* spp. and response to inoculation varied with species (Table II). The concentrations and contents of reducing and non-reducing sugars were generally higher in inoculated bulbs than non-inoculated bulbs, and response to inoculation did not vary with species or soil pasteurization treatment (Table II).

TABLE II

Influence of soil pasteurization and inoculation with the VAMF Glomus intraradices *on protein, amino acid, and carbohydrate concentration and content of bulbs from three* Zephyranthes *spp. at the end of the first growing cycle after treatment*

		Concentration (g kg^{-1})			
Species[z]	Treatment	Protein	Amino acid	Reducing sugar	Non-reducing sugar
WRL	NPS+NONE	3.80 c[x]	23.20 c	7.09 a	6.21 a
	NPS+VAMF	3.31 b	14.60 a	7.93 b	7.12 b
	PS+NONE	3.36 b	19.80 b	8.07 b	6.51 a
	PS+VAMF	3.05 a	14.10 a	10.52 c	8.16 c
YZL	NPS+NONE	3.04 a	25.20 c	7.05 a	6.90 a
	NPS+VAMF	2.78 a	18.40 b	8.64 b	8.01 b
	PS+NONE	4.38 c	17.20 b	9.40 b	8.34 b
	PS+VAMF	3.93 b	12.30 a	10.78 c	9.39 c
PFL	NPS+NONE	3.92 b	18.10 c	7.69 a	6.91 a
	NPS+VAMF	3.09 a	12.60 a	9.67 b	7.89 b
	PS+NONE	3.97 b	16.60 b	9.56 b	8.71 c
	PS+VAMF	3.83 b	12.10 a	11.42 c	9.74 d
(SE)[w]		(0.21)	(0.42)	(0.36)	(0.24)
ANOVA Effects (P<0.05)[v]		Z, V, Z*S	Z, S, V, Z*S, Z*V, S*V	Z, S, V	Z, S, V
		Content (mg bulb^{-1})			
Species	Treatment	Protein	Amino acid	Reducing sugar	Non-reducing sugar
WRL	NPS+NONE	35.3 b	218.6 d	66.5 a	57.9 a
	NPS+VAMF	40.8 c	177.2 b	95.9 c	83.5 c
	PS+NONE	33.3 b	191.8 c	78.4 b	62.3 a
	PS+VAMF	26.2 a	123.1 a	91.1 c	71.4 b
YZL	NPS+NONE	26.4 a	220.5 d	60.9 a	60.1 a
	NPS+VAMF	27.3 a	178.8 b	83.9 b	80.5 b
	PS+NONE	53.0 c	207.3 c	119.2 c	100.6 c
	PS+VAMF	42.8 b	132.8 a	115.9 c	101.1 c
PFL	NPS+NONE	36.9 c	171.9 d	74.3 c	76.4 b
	NPS+VAMF	37.8 c	157.4 c	122.8 d	110.1 b
	PS+NONE	30.6 b	85.6 b	49.9 a	45.8 a
	PS+VAMF	19.4 a	62.5 a	58.1 b	49.6 a
(SE)		(3.4)	(14.9)	(9.4)	(8.3)
ANOVA Effects (P<0.05)		Z, S, Z*S	Z, S, V	Z, S, V, Z*S	Z, S, V, Z*S

[z]WRL = White Rain Lily (*Z. candida*), YZL = Yellow Zephyr Lily (*Z sulphurea*), PFL = Pink Fawn Lily (*Z. robusta*).
[y]NPS = Non-pasteurized soil; PS = Pasteurized soil; NONE = Sterilized VAMF inoculum, VAMF = VAMF inoculum.
[x]Means followed by the same letter within a species are not significantly different (P<0.05) using single degree of freedom contrasts.
[w]SE across all species (n = 6).
[v]Significant (P<0.05) main effects and interactions from ANOVA where Z = Plant species; S = Soil pasteurization treatment, V = VAMF inoculation treatment, Y = year from inoculation.

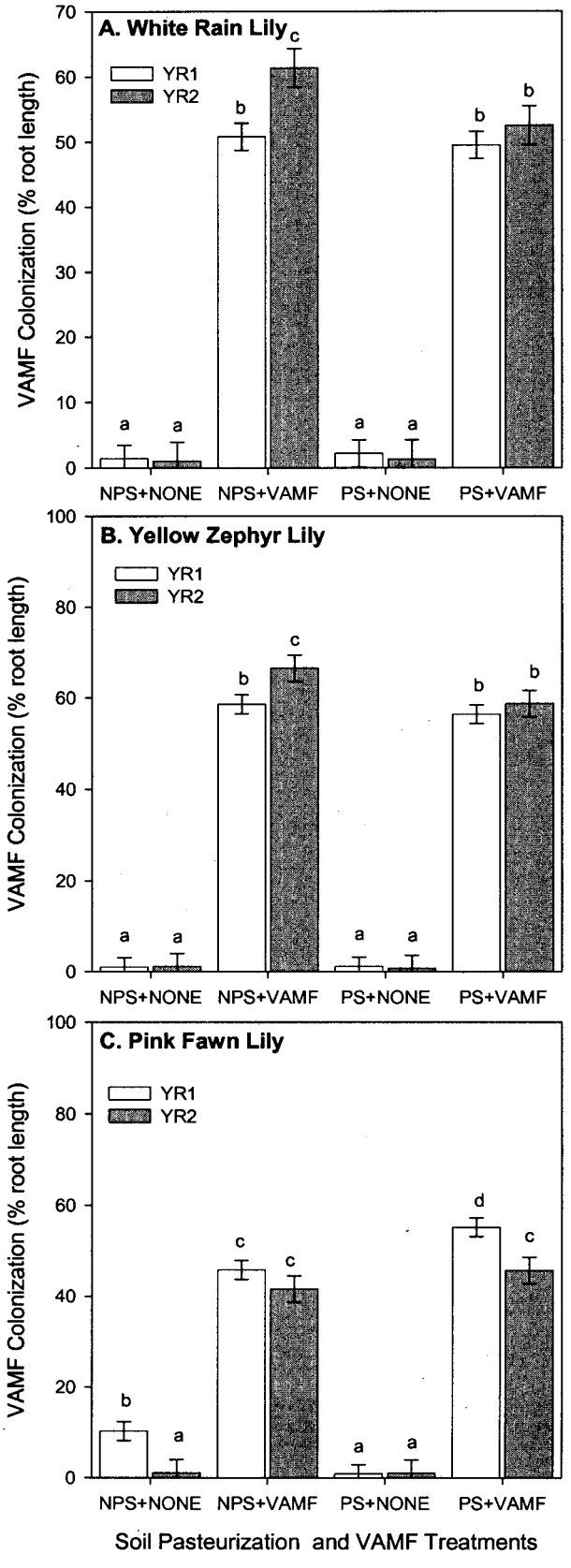

FIG. 8
Influence of soil pasteurization and inoculation with the VAMF *Glomus intraradices* on root colonization by VAMF (A–C) of three *Zephyranthes* spp. during the first growing cycle after inoculation and soil treatments. (A) White Rain Lily, (B) Pink Fawn Lily, and (C) Error bars are SEs of the least squares means within measurement dates (YR1, n = 12, YR2, n = 6). Columns with the same letters above them within a species are not significantly different ($P<0.05$).

Root colonization

Root colonization of non-inoculated plants was less than 8% of total root length at the time of harvest, irrespective of soil pasteurization treatment (Figure 8A-C). Inoculation significantly increased colonization over background levels of colonization found in non-inoculated plants in both pasteurized and non-pasteurized soil, and the magnitude of response to inoculation varied with species and year from inoculation. Root colonization of inoculated WRL and YZL increased slightly between the first and second growing cycles, while root colonization of inoculated PFL decreased slightly between the first and second growing cycles (Figure 8A-C). Soil pasteurization had no effect on colonization.

DISCUSSION

Liliaceous plants are important in a wide variety of horticultural systems used for the production of food and ornamental crops. The beneficial effects of VAMF on the growth and nutrition of onion has been well documented in many experimental systems designed to investigate the mechanisms involved in the interaction between the fungus and plant. The influence of VAMF on liliaceous plants that are used in the production of cut flowers and ornamental crops has not been investigated to a similar depth. Overall, we found that inoculation of different *Zephyranthes* spp. with VAMF alters aspects of plant development important to flower and bulb production, and responses to VAMF varied with cultivar and soil pasteurization.

In production of potted flowers, decreasing the time required to reach marketable size can decrease production costs. We found that VAMF inoculation generally decreased the number of days until shoot emergence of all three *Zephyranthes* spp. tested (Figure 1). In contrast, inoculation with VAMF delayed shoot emergence of *Brodiaea laxa* (Scagel, 2003) and *Lilium longiflorum* (Mora, 1990) when grown in pasteurized soil. Earlier shoot emergence of VAMF inoculated *Zephyranthes* spp. occurred in both years of the study, indicating that VAMF may have also altered aspects of bulb quality that influenced shoot emergence the second growth cycle following inoculation. VAMF promotion of shoot emergence may also play a role in carbohydrate status of the plants by extending the period of photosynthetic activity in the geophytic life cycle. In other plants, VAMF colonization has been shown to increase photosynthetic activity and hasten leaf appearance (Eissenstat *et al.*, 1990) and may be an adaptive response of the plant to the increased carbohydrate demands resulting from VAMF colonization. In the *Zephyranthes* spp. tested in our study, however, even though shoots of inoculated plants emerged earlier than those of non-inoculated plants the total number of leaves produced per plant was not consistently increased by inoculation (Figure 5) and leaf dry weight of YZL and PFL was decreased by inoculation (Figure 4). This suggests that the photosynthetic area of the VAMF inoculated plants may have been different from that of non-inoculated plants. Further analysis of photosynthetic efficiency and leaf area development in response to VAMF inoculation in *Zephyranthes* would allow for a better understanding

of the role of these fungi in carbon partitioning and efficiency of vegetative growth during production.

In *Zephyranthes* spp., flower initiation and growth occurs within the same year. Once flower initiation starts, floral parts are formed within the first 3–4 weeks (De Hertogh and Le Nard, 1993). Differences in resource allocation during flower initiation resulting from VAMF colonization have the potential to delay flowering. In our study, the number of days between shoot emergence and flower bud emergence was generally greater in VAMF inoculated WRL and PFL (Figure 2D and F). This delay in emergence of flower buds of WRL and PFL could be a result of different resource allocation patterns in VAMF-inoculated plants during early stages of colonization (Koide, 1985). In fact, flowers on WRL opened at the same time regardless of inoculation, indicating that the delayed flower bud emergence in VAMF inoculated WRL did not significantly affect the time of flowering (Figure 3A and D). In contrast, VAMF inoculation delayed flower opening after shoot emergence of PFL by approximately 12 d (Figure 3F) and when YZL bulbs were inoculated with VAMF, flower buds emerged (Figure 2B and E) and opened (Figure 3B and E) earlier than on non-inoculated plants. In most geophytes, the major factor controlling flowers is seasonal thermoperiodicity (Han *et al*., 1991). Mora (1990) found that inoculation of *L. longiflorum* with *G. intraradices* did not significantly affect flower emergence, although flower emergence varied with nitrogen type used in the fertilizer. The earlier flowering of inoculated YZL in our study suggests that any changes in resource allocation, resulting from the establishment of the symbiosis, did not negatively affect flower development and actually hastened development. Inoculation with VAMF has also been reported to decrease the time until flowering of *B laxa* (Scagel, 2003). The effects of VAMF on flower development of the three different *Zephyranthes* tested in our study may be a result of genetic differences in carbon partitioning and demands of different species. For instance, PFL produced flowers that were much larger than those of either YZL or WRL. VAMF inoculation may have more influence on altering carbon partitioning during flower development of PFL than YZL or WRL as a result a higher demand for carbon based on flower size.

In *Zephyranthes* spp., flower formation generally occurs alternately with leaf formation during the whole assimilation period (De Hertogh and Le Nard, 1993). Colonization of roots by VAMF has been shown to cause differences in plant biomass partitioning (Smith and Read, 1997). We found that inoculation with increased dry weight of stems and leaves of WRL (Figure 4D), however the number of leaves and flowers was generally not affected by inoculation (Figure 5A). We also found that leaf production on WRL generally stopped as soon as flower production started. This indicates that VAMF-inoculated WRL produced larger leaves in response to the carbon demand of the symbiotic relationship, but did not alter carbon allocation for flower production. In contrast, PFL had smaller leaves (lower leaf biomass and more leaves) (Figure 4F, Figure 5C) and delayed flower production when compared with non-inoculated plants. VAMF inoculation of YZL also resulted in plants with smaller leaves than those on non-inoculated plants (Figure 4E, Figure 5B), however flower production was not delayed. We also found that leaf production in both PFL and YZL continued after the start of flower production. These results indicate that when growing in non-pasteurized soil, inoculation of PFL and YZL with VAMF altered patterns of carbon allocation in the aboveground portion of the plant. The species-specific differences in allocation patterns resulting from VAMF inoculation may be a result of differences in synchronization between leaf and flower development.

Inoculation of plants with VAMF significantly increased the number of flowers produced per plant but only when plants were grown in pasteurized soil (Figure 5D, E, and F). Although flowers expanded earlier on inoculated PFL than on non-inoculated plants, the total number of flowers produced was only increased by VAMF when plants were grown in pasteurized soil (Figure 5A, B). Inoculated YZL produced more flowers than non-inoculated plants, regardless of soil pasteurization treatment (Figure 5A, C). For all species, plants stopped producing new flowers at approximately the same time for all soil pasteurization and VAMF treatments. Inoculation with the VAMF *G. intraradices* has also been reported to increase the number of flowers on *B. laxa* growing in pasteurized and non-pasteurized soil (Scagel, 2003). In contrast, Mora (1990) found that inoculation of *L. longiflorum* with *G. intraradices* did not significantly affect flower production on plants growing in pasteurized soil.

Commercial production of *Zephyranthes* spp. is generally done from offset bulblets (De Hertogh and Le Nard, 1993). In the first year of our study, VAMF inoculation generally had no influence on daughter bulb biomass, partitioning between daughter bulbs and offsets, offset size, or number of offsets produced. At the end of the second growing cycle VAMF inoculation increased the combined weight of bulbs and offsets by between 50–150% depending on species and soil pasteurization treatment. Inoculated WRL and YZL had higher biomass partitioning to offsets and produced more offsets by the end of the second growing cycle while inoculation had no effect on partitioning between daughter bulbs and offsets of PFL (Figure 6). In contrast, inoculation with the VAMF, *G. intraradices*, has been reported to produce smaller cormels and increase daughter corm biomass and partitioning to daughter corms over cormels of *B. laxa* for two growing cycles after inoculation (Scagel, 2003). Charron *et al.* (2001) found that onion bulbs reached marketable size 2–3 weeks earlier when inoculated with VAMF. In *Zephyranthes* spp., most gains in bulb weight accumulate before anthesis (De Hertogh and Le Nard, 1993), therefore it is possible that increased flower production in response to VAMF inoculation could have played a role in reducing average offset weight. Changes in biomass partitioning and offset size in response to VAMF inoculation may have a positive or negative influence the multiplication rate of *Zephyranthes* spp. by offset production. With WRL and YZL, VAMF inoculation during production for offsets may be beneficial for increasing offset size.

Increased uptake of elements in response to VAMF inoculation has frequently related to plant growth responses to inoculation (Smith and Read, 1997). Increased uptake of certain elements by geophytes has been associated with disease tolerance and dormancy (De Hertogh and Le Nard, 1993). We found that the concentrations and contents of P, K and Zn in *Zephyranthes* spp. bulbs at the end of the first growing cycle were generally higher in VAMF than non-VAM inoculated plants while N content and concentrations in bulbs of VAMF-inoculated plants was generally lower or equal to that found in non-inoculated plants (Table I). Increased P, K, and Zn content and concentration along with increased bulb weight indicates that VAMF increased the uptake, availability, or storage of these elements in bulbs. Enhanced acquisition of these elements also has been reported for various plants colonized with different VAMF isolates compared with non-VAM plants (Clark and Zeo, 2000). In our study, increased bulb weight accompanied by decreases in both N content and concentration suggest that VAMF inoculation increased the efficiency of N use (e.g. more growth given less nutrient in plant) in *Zephyranthes* spp. In contrast, VAMF colonization of *B. laxa* was found to enhance uptake and corm storage of K, Zn, and N but not P (Scagel, 2003). It is possible that these VAMF induced changes in uptake and use of elements in *Zephyranthes* spp. may have beneficial influences on the storage quality of bulbs and may play an indirect role in the difference observed in plant growth, bulb production and flowering between inoculated and non-inoculated plants.

Organic carbon and nitrogen stored in bulbs at the end of a growing cycle are important for growth during the following growing cycle when storage reserves in mother bulbs are depleted by new growth. In developing storage organs such as bulbs, translocated photoassimilates are converted into carbon and nitrogen reserves such as starch, fructans, oil, and storage proteins (Kavakli *et al.*, 2000). In bulbs of *Zephyrantes* spp., scales and leaf bases are the primary storage areas (De Hertogh and Le Nard, 1993). We found that inoculation with VAMF decreased protein and amino acid concentrations in bulbs (Table II), but changes in protein and amino acid concentration were associated with proportional increases in protein and amino acid content and bulb weight, therefore inoculation had no influence on the production and accumulation of protein and amino acids in bulbs. Similar changes in protein and amino acid content and concentrations in response to VAMF inoculation have also been reported for corms of *B. laxa* (Scagel, 2003). In contrast, Vazquea *et al.* (2001) found that VAMF can increase root and shoot protein content, however, responses depended on the species of VAMF used. In our study, and in that reported in Scagel (2003) the same isolate of VAMF was used for inoculations. It is possible that other VAMF species or isolates may have a different influence on the protein content of bulbs. In some geophytes, amino acids have been found to be low at harvest and increase with heat (Le Nard *et al.*, 1988). Others (Krishna and Bagyaraj, 1983) have reported that VAMF increases accumulation of amino nitrogen in roots; however these studies were done with different VAMF isolates and on annual plant species that do not accumulate storage reserves in a similar manner as geophytic species.

The differences in biomass partitioning we observed in response to VAMF inoculation of the three *Zephyranthes* spp. suggests VAMF inoculation can alter aspects of carbon metabolism during the growing cycle that may influence carbohydrate storage. We found that the concentrations and contents of reducing and non-reducing sugars were generally higher in inoculated bulbs than non-inoculated bulbs of *Zephyranthes* spp. (Table II), and response to inoculation did not vary with species or soil pasteurization treatment. Increased concentrations and content of carbohydrates, along with increased bulb biomass, indicates that VAMF inoculation increased carbohydrate production and storage in bulbs. In contrast, inoculation of *B. laxa* increased production and accumulation of non-reducing sugars in corms but had no effect on reducing sugars (Scagel, 2003). It is possible that the increased storage of carbohydrates in bulbs of *Zephyranthes* spp. after the first growing cycle in response to VAMF inoculation may be related to the more pronounced VAMF-effects we observed on plant growth and offset production during the second growing cycle. This suggests that carbohydrate storage may play a major role in the responsiveness of *Zephyranthes* spp. to VAMF inoculation.

The extent of root colonization by mycorrhizal fungi has been related to several plant growth responses to inoculation (Smith and Read, 1997). In our study, root colonization of non-inoculation *Zephyranthes* spp. was less than 8% of total root length at the time of harvest, irrespective of soil pasteurization treatment (Figure 8A-C). Inoculation significantly increased colonization over background levels of colonization found in non-inoculated plants in both pasteurized and non-pasteurized soil, and the magnitude of response to inoculation varied with species and year from inoculation. Under the cultural conditions of our experiment, 40–60% of the total root length of the inoculated *Zephyranthes* spp. showed signs of colonization by the end of the growing cycle. Others have reported that VAMF colonization peaks at mid-bulb filling stage in garlic (Al-Karaki, 2002). It is possible that levels of root colonization measured at the end of the growing cycle in our experiment are not representative of the highest levels of colonization present during the rest of the growing cycle. In our study, soil pasteurization had no effect on colonization, suggesting that the level of VAMF inoculum in the soil used in our study was low and that the differences in plant responses to colonization between pasteurized and non-pasteurized soil were not related to the level of inoculum or level of colonization. Soil contains populations of organisms that can have beneficial or detrimental effects on plant growth and productivity. Cultural treatments used to control detrimental organisms may not only influence the presence of natural populations of mycorrhizal fungi in soil, but also influence the effects of plant inoculation with VAMF. Vosátka (1995) found that the growth responses of onion to inoculation were higher when indigenous fungi in the soil were eliminated by steam sterilization. In our experiment, colonization of inoculated plants was not

influenced by soil pasteurization, however biomass of leaves, bulbs and offsets were generally decreased by soil pasteurization and flower production was increased. This indicates that there are may be soil organisms, other than mycorrhizal fungi, than can have beneficial effects on the growth and productivity of *Zephyranthes* spp. similar to the responses described by Linderman (1988). Another possibility is that soil pasteurization altered the chemical composition in the soil causing the release of compounds toxic to plant growth, e.g. manganese (Wolf *et al*., 1989).

Maximum productivity and quality of geophytes used for both flower and corm production requires a balance between resources allocated to flowering and corm production. The symbiotic relationship between VAMF and their plant symbiont can alter aspects of biomass partitioning and metabolism that differentially influence productivity and quality of *Zephyranthes* spp. When growing *Zephyranthes* for in pot culture for cut flower or potted plants, our results indicate that adding VAMF inoculum into the growing medium can alter aspects of flowering and biomasses partitioning that are important in the commercial production of this crop for cut flowers and corms. Inoculation may delay flower emergence and decrease leaf production in some *Zephyranthes* spp., and hasten flower emergence, and increase the number of flowers, leaves, and offsets produced in other species. The effects of inoculation with VAMF on responsive *Zephyranthes* species are more pronounced in the second growing cycle after inoculation. Species-specific responses of *Zephyranthes* to inoculation with VAMF and soil pasteurization treatments suggests that combinations of species, cultural practices, and VAMF inocula should be tested before VAMF inoculum is used on a large scale across all species of this crop.

The author gratefully acknowledges the technical assistance of Kathleen Eggemeyer, Jesse Mitchell, Lisa Tribbet, and Benjamin Jackson. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Dept. of Agriculture and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

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